

REVIEW ARTICLE

Models of T cell antigen receptor activation: the puzzle still remainedSumit Deswal^{a,b}^aDepartment of Molecular Immunology, Max Planck Institute of Immunobiology and Epigenetics and Faculty of Biology III, University of Freiburg, Stübeweg 51, 79108 Freiburg, Germany^bSpemann Graduate School of Biology and Medicine (SGBM), University of Freiburg, GermanyCorresponding address: Max Planck Institute of Immunobiology and Epigenetics, Stübeweg 51, 79108 Freiburg, Germany. E-mail: deswal@immunbio.mpg.de, Tel.: +49-761-5108-314, FAX: +49-761-5108-423**Abstract**

T cell antigen receptor (TCR) is a protein-complex expressed on all T cells of the immune system and is responsible for the activation of T cells when infectious agent is presented by an antigen presenting cell (APC) in the form of peptides bound to the major histocompatibility complex (pMHC). Despite numerous studies it is not clear what biochemical changes upon binding of antigen ligand to the extracellular domains of TCR leads to activation of intracellular signaling (a process known as TCR triggering). This review summarizes possible biochemical mechanisms for TCR triggering and discusses their comparative limitations and advantages in explaining various experimental observations.

Keywords: T cell antigen receptor, activation, model**Introduction**

T cell antigen receptor (TCR or TCR-CD3) is a protein complex expressed exclusively in T cells and is composed of the variable TCR α and β chains and the constant CD3 γ , CD3 δ , CD3 ϵ and CD3 ζ chains (Figure 1). CD3 γ and CD3 δ chain are glycoproteins each of which form a heterodimer with non-glycosylated CD3 ϵ chain. Along with the CD3 ζ - ζ homodimer, these chains associate with the

TCR $\alpha\beta$ heterodimer to generate the full TCR-CD3 complex [1]. The TCR-CD3 is responsible for activation of a T cell upon antigen encounter which then help in the activation of B cells by releasing helper cytokines (helper T cells) or kill the target cell directly by the secretion of cytotoxic effector molecules such as granzymes, perforin and granulysin.

Antigenic ligand binding to the TCR leads to the phosphorylation of immunoreceptor

tyrosine-based activation motifs (ITAMs) of the CD3 complex by the Src family protein tyrosine kinase Lck, resulting in the membrane targeting and activation of another kinase, ZAP-70. Once activated, ZAP-70 phosphorylates several substrates, including the transmembrane adaptor protein, linker for activation of T cells (LAT). Phospho-LAT serves as an important point of divergence for signals initiated from the TCR by recruiting several effector molecules to the plasma membrane thus initiating multiple pathways essential for full T cell activation [2].

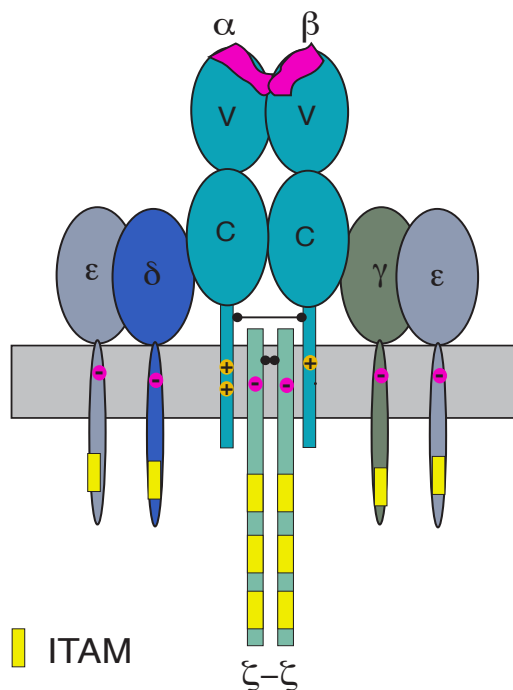


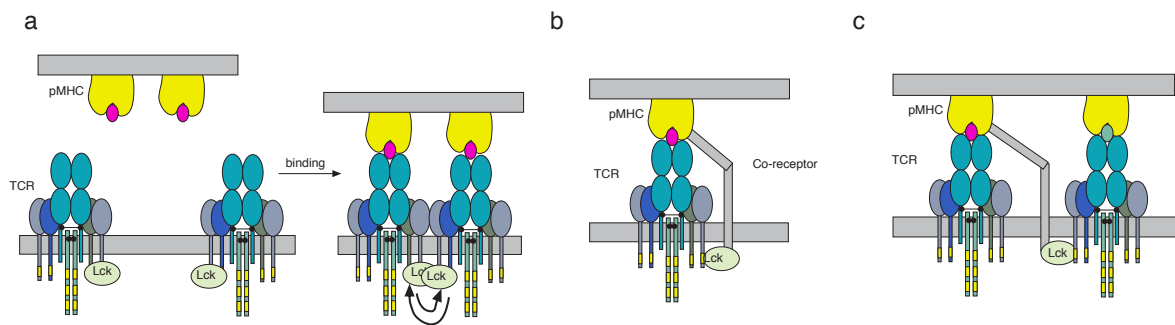
Fig.1: The TCR-CD3 complex. V and C represent variable and constant domains respectively for the TCR α and β . The hypervariable region that binds to the pMHC complex is shown in red color.

Although the downstream signaling mechanisms of T cell activation have been explored to quite detailed extent, the basic mechanism of how ligand binding to extracellular domains of TCR $\alpha\beta$ leads to downstream signaling remained unclear. Several models of TCR triggering have been proposed based on the experimental findings, but none of the models so far could explain every aspect of TCR triggering. Broadly, these models can be grouped in three major processes which involve aggregation, conformational change or segregation (Figure 2). This review summarizes the proposed models of TCR triggering and discusses their comparative abilities for explaining various experimental observations (Table 1).

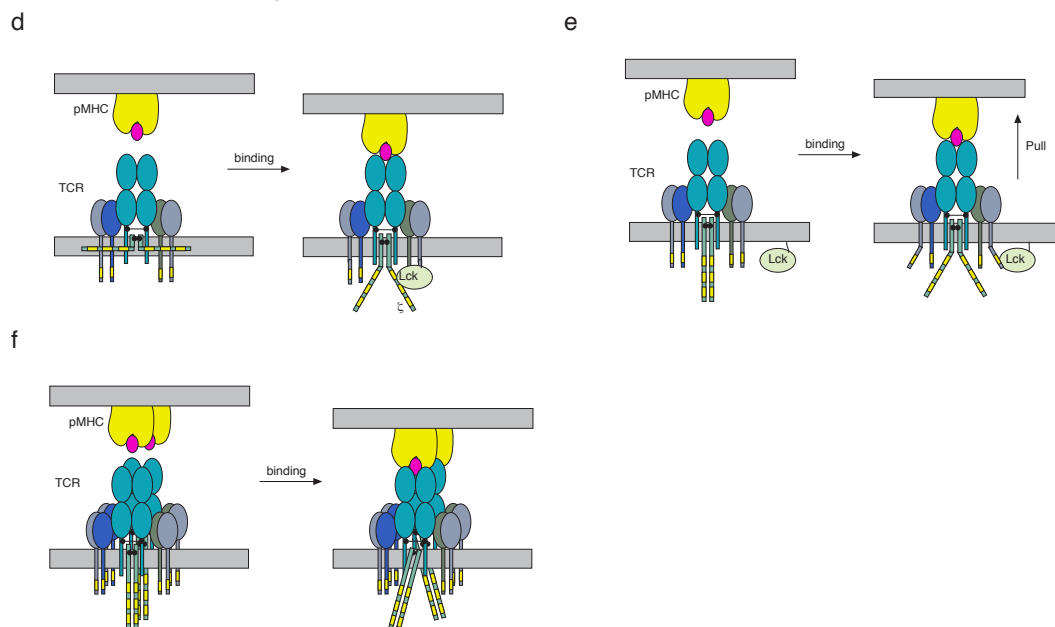
1. Antigen induced clustering

Several forms of clustering have been proposed as the triggering mechanism for the TCR. These include homodimer, heterodimer and the pseudodimer models as discussed below.

Clustering models



Conformational change models



Segregation models

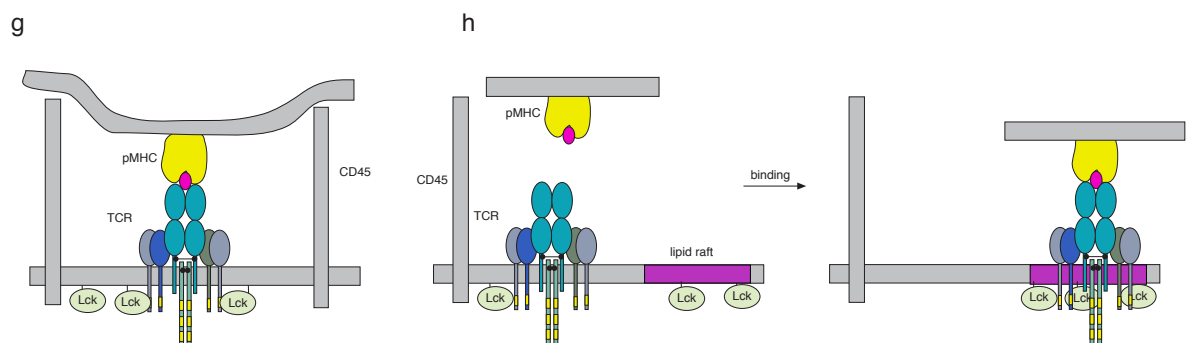


Fig.2: Various models of TCR triggering. a) Homodimer model, b) Heterodimer model, c) Pseudo-dimer model, d) Membrane binding model, e) Kinetic deformation model, f) Permissive geometry model, g) Kinetic segregation, h) Lipid raft mediated segregation model. Full description of each model is given in the main text.

Table 1: Abilities of different models to explain various experimental observations.

Observation	Triggering model							
	Homodimer	Heterodimer	Pseudo-dimer	Membrane binding	Kinetic deformation	Permissive geometry	Kinetic segregation	Lipid raft
Pre-clustered TCR	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Coreceptor independent triggering	Yes	No	No	Yes	Yes	Yes	Yes	Yes
Triggering by soluble monomer pMHC	No	Yes	No	Yes	No	No	No	Yes
Triggering by anti-CD3 antibodies	Yes	No	No	Yes	No	Yes	No	No
Activation by pervanadate	No	No	No	No	No	No	Yes	Yes
Inhibition by truncated CD45	No	No	No	No	No	No	Yes	No
Inhibition by elongated pMHC	No	No	No	No	No	No	Yes	No
Triggering in absence of self pMHC	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes

a. Homodimer model

Clustering of TCR–CD3 complexes following TCR engagement could lead to enhanced phosphorylation, for example by increasing the proximity of associated Lck molecules, resulting in the activation of the second receptor in the cluster by *trans*-autophosphorylation (or this enhanced proximity of kinases might lead to their trans-phosphorylation which enhance their activity to phosphorylate the receptor). This model is

supported by the observations that artificial ligands such as pMHC tetramer and the anti-TCR $\alpha\beta$ and anti-CD3 antibodies that crosslink the TCR lead to its activation whereas Fab fragment of these antibodies or the monomeric pMHC in solution fail to activate the TCR [3]. Also the observations that MHC class I [4, 5] and MHC class II [6] exist in pre-formed clusters, support the homodimer model. However, since several studies have now shown the existence of pre-clustered TCRs on the T cell surface [7, 8], it is difficult to imagine that clustering alone could trigger the

TCR. In fact in a study by Schamel and colleagues [9], both clustering and conformational change were shown to be required for TCR triggering (permissive geometry model, as discussed later).

b. Heterodimer model

In the heterodimer model [10], coreceptor CD8 or CD4 binding to the same pMHC complex as the TCR brings the coreceptor-associated Lck kinase into proximity with TCR-CD3 ITAMs and their phosphorylation. Further it has been proposed that to fully activate the TCR, a pMHC ligand need to interact with a TCR-CD8 pair with a threshold time to induce stable zippering between the membrane-proximal domain of CD8 and the connecting peptide motif in the TCR α [11]. This allows stable association of Lck with the CD3 complex and results in complete phosphorylation of the CD3 ITAMs. A low-affinity pMHC ligand that interacts with a TCR-CD8 pair with less than threshold time induces incomplete zippering and therefore allows only transient Lck association and partial CD3 phosphorylation. However, the observations that T cells can develop and function normally in the mice lacking both CD4 and CD8 suggest that coreceptors are not absolutely required for TCR triggering and T cell activation [12, 13]. Also the fact that anti-

CD3 antibodies and their F(ab')₂ fragments that do not engage the co-receptors can still activate the TCR [14, 15], indicates that co-receptors are not required for the TCR triggering itself, though they might be important for full activation of a T cell.

c. Pseudo-dimer model

Only a few MHCs carry high affinity agonist peptides for the TCRs, whereas a great majority of MHCs carry endogenous self-peptides. On the basis of the crystallographic studies which showed that the CD4 tail associating with Lck was far from their own TCR/CD3 complex, the pseudodimer model postulates that two TCRs are brought together by binding low-affinity self or high-affinity agonist pMHC ligands and that the CD4 associated with a TCR engaging the agonist pMHC complex can assist in phosphorylation of neighboring TCR (engaged to low affinity self peptide) by the associated kinase Lck [16]. Thus according to this model, a 'pseudodimer' consists of one foreign and one self-antigen engaged receptor linked via CD4 molecule is the primary unit of TCR signal initiation module.

2. Conformational change model

Several studies have proposed conformational

change as a requirement for TCR triggering [17, 18]. However, how the ligand binding to TCR $\alpha\beta$ ectodomains leads to a conformational change in the cytoplasmic tails of CD3 subunits is not clear. The situation is complicated by the fact that the crystal structure of the fully assembled TCR-CD3 complex is not known, although individual subunits have been crystallized partially [19-22]. Some of the important models for the mechanism of conformational change in TCR-CD3 are discussed below.

a. Membrane binding model

According to this model, cytoplasmic tails of CD3 ϵ [23, 24] and CD3 ζ [25] associate with the lipids present in the plasma membrane of a T cell. Thereby, the ITAMs are buried in the plasma membrane, making them inaccessible for phosphorylation by the kinases. Ligand binding leads to release of these CD3 chains from the plasma membrane, making them accessible to kinase and hence the phosphorylation.

b. Kinetic deformation model

Several line of evidence have proposed that the mechanical effects (such as pulling or

shearing) of pMHC binding to the TCR leads to a piston-like displacement of the TCR-CD3 complex [26, 27]. This induces a change in the conformation of the CD3 cytoplasmic domains, allowing ITAM phosphorylation.

c. Permissive geometry model

Based on the experimental evidence for the existence of TCR-CD3s in a pre-clustered form [7] and the fact that pMHC monomers in solution fail to trigger TCR [28, 29] while pMHC dimer or higher oligomer can induce TCR triggering, the permissive geometry model was proposed [30]. According to this model, before ligand binding, the mono and multivalent TCR-CD3s exist in an autoinhibited state where ITAMs are inaccessible for phosphorylation by the kinase. Simultaneous dimeric (or higher order) ligand binding to the two TCR-CD3 complexes leads to a scissor-like movement in the two TCR-CD3 complexes leading to exposure of their cytoplasmic tails, thus making the ITAMs accessible for phosphorylation.

3. Segregation Model

In a resting T cell, phosphorylation of the TCR-CD3 is kept in check by the active phosphatases. This is supported by the fact that pervanadate, a phosphatase inhibitor,

treatment leads to strong phosphorylation of the TCR-CD3 ITAMs and also initiates the downstream signaling [31]. Thus any process that favors the kinase-phosphatase balance towards kinase, can lead to TCR-CD3 phosphorylation and the signaling. Redistribution of the TCR-CD3, kinases and phosphatases in which phosphatase is segregated from the TCR can interfere with the dephosphorylation. There are two mechanisms proposed for such ligand dependent re-distribution, which are discussed below.

a. Kinetic segregation

Kinetic segregation model was proposed based on the topological view of the cell surface molecules at the T cell–APC interface [32, 33]. The tight intercellular contact causes the segregation of the molecules by sizes of their ectodomain resulting in physical separation of TCR from the large inhibitory tyrosine phosphatase CD45, leading to stable phosphorylation of TCR–CD3 ITAMs by Lck. Though sounding good in the context of a T-cell – APC contact, the model fails to explain the activation of T cells by soluble anti-TCR $\alpha\beta$ and anti-CD3 antibodies and the pMHC tetramer [14, 34].

b. Lipid raft mediated segregation model

Lipid rafts are the detergent (such as Triton X-100, Brij-series, NP-40 or CHAPS)-resistant membrane microdomains which are enriched in glycosphingolipids, cholesterol and lipid-modified proteins such as the GPI-anchored proteins. Also these microdomains are enriched in double-acylated (myristoylated, palmitoylated) Src-family kinases and the important signaling molecules such as the coreceptors CD4 and CD8 and the adaptor protein LAT. Lipid raft mediated segregation model postulates that pMHC engagement results in partitioning of the TCR–CD3 complex into lipid rafts enriched in Lck and deficient in CD45 [35, 36]. Partial support for this model comes from the observations that palmitoylation-deficient (and raft excluded) mutants of Lck and LAT are functionally defective. In addition, artificial targeting of other cytoplasmic molecules, such as SHP-1 [37], CD45 [38] or PLC γ [39], to membrane rafts has marked functional effects on TCR-induced signaling. A connecting peptide in the TCR α chain and the CD3 δ chain were identified as the critical sites essential for effective raft association. Mutations in these components interfere with TCR signaling [40]. The mechanism of directing association of the engaged TCR complex with lipid rafts is not clear but might be based on co-engagement of the raft resident CD4/CD8 coreceptors.

Conclusion

Analysis of various proposed mechanisms reveals that probably a combination of these mechanisms is responsible for TCR triggering. Existence of pre-clustered TCR molecules rules out the homodimerization as a requirement for TCR triggering. It is possible that CD3 ITAMs are inaccessible for phosphorylation by the kinase in the resting state and some sort of conformational change is necessary for exposing the ITAMs, but additional studies are required to find out what mechanism is responsible for such conformational change. Segregation models argue the balance of kinase mediated phosphorylation and phosphatase mediated dephosphorylation shifts in favour of kinase upon ligand binding. Though such segregation might contribute for TCR triggering in the context of a T cell-antigen presenting cell interaction, it seems not the sole mechanism of TCR triggering as the soluble anti-TCR or anti-CD3 antibodies which do not induce segregation can still cause TCR triggering. Further studies on the structure of fully assembled TCR-CD3 complex will be required for a better understanding of the TCR triggering.

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